DATA EVALUATION RECORD
§ 72-2 -- ACUTE LC_{50} TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** Methyl Bromide PC Code No.: 053201

2. **TEST MATERIAL:** Methyl Bromide Purity: 99.87%

3. **CITATION**

Authors: Drottar, K.R. and J.P. Swigert
Title: Methyl Bromide: A 48-hour Static Acute Toxicity Test with the Cladoceran (Daphnia magna).
Study Completion Date: July 31, 1993
Laboratory: Wildlife International Ltd., Easton, MD
Sponsor: Methyl Bromide Industry Panel
Chemical Manufacturers Association
Laboratory Report ID: 264A-102B
MRID No.: 429329-01
DP Barcode: D197112

4. **REVIEWED BY:** (Tom A. Bailey), (Fishery Biologist), EEB, EFED

Signature: Tom A. Bailey **Date:** April 18, 1995

5. **APPROVED BY:** (Henry T. Craven), Head of Section (4), EEB, EFED

Signature: Henry T. Craven **Date:** 4/18/95

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: (*Daphnia magna*)
Age of Test Organism: 1st instar <24 hours old
Definitive Test Duration: 48 hours
Study Method: Static (sealed serum bottoms with septa)
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** The study was scientifically sound and meets guideline requirements a 72-2 acute freshwater invertebrate toxicity study. On the basis of mean measured concentrations, the 48-hour EC50 as determined by the binomial method was 2.6 mg a.i./L, which classifies methyl bromide as moderately toxic to *Daphnia magna*. The No Observable Effects Concentration was 1.2 mg a.i./L.

Results Synopsis

LC_{50}: 2.6 ppm ai 95% C.I.: 2.2 - 3.5 ppm ai
NOEL: 1.2 ppm ai Probit Slope: ____

8. **ADEQUACY OF THE STUDY**

2010700

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

9. BACKGROUND

Because of the volatile nature of methyl bromide, special precautions and procedures were necessary. Subsamples of the purchased test material were removed and stored at -14 C. The remaining test substance was maintained at 4 C. Test concentrations were prepared directly into test containers on a weight basis. Evacuated Tedlar bags were filled with liquid methyl bromide, the methyl bromide was allowed to vaporize, methyl bromide was removed form the Tedlar bags with gas-tight syringes, and known quantities of methyl bromide were transferred to 100 mL air-tight serum bottles (covered with septa) filled with known weights of serum water.

10. GUIDELINE DEVIATIONS

1. The Brood number of test daphnids were not reported.

11. SUBMISSION PURPOSE: This study was submitted pursuant to an Agency Data-Call-In. The objective of the test is to determine the acute toxicity of methyl bromide to a cladoceran, *Daphnia magna* under static conditions.

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is *Daphnia magna*	*Daphnia magna*
All organisms are approximately the same size and weight?	Not Reported
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3rd instar.	1st instar

Guideline Criteria	Reported Information
Supplier	Cultures maintained by Wildlife International, Easton Maryland
All organisms from the same source?	Yes

B. **Source/Acclimation**

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	14 days?
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	(N/A)
Feeding No feeding during the study.	(Adults fed prior to test initiation)
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	0% mortality prior to testing

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water.	Well water (45 meters deep) Medium-hard water source
Does water support test animals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20°C
pH 7.6 to 8.0 is recommended.	8.1
Dissolved Oxygen Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%.	(lowest=89% DO & 48-hour)
Total Hardness 160 to 180 mg/L as $CaCO_3$ is recommended.	144 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 ml (daphnids and midges) or 2-3 L.	1. glass 2. 100 mL 3. 125 mL
Type of Dilution System Must provide reproducible supply of toxicant.	(N/A)
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	_ vol/24 hours (N/A)
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.	5 daphnids per test chamber
Photoperiod 16 hours light, 8 hours dark.	16 hours light, 8 hours dark. 30 minute transition period.

Guideline Criteria	Reported Information
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	(N/A)

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L, then no definitive test is required.	Results less than 100 mg/L
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	1.3, 2.2, 3.6, 6.0, 10 mg/L
Number of Test Organisms Minimum 20/level, may be divided among containers.	20 organisms/level
Test organisms randomly or impartially assigned to test vessels?	Yes
Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	Temperature measured continuously in surrogate negative control; ranged from 19.5 to 20 C. pH measured in surrogate of each treatment at test initiation, in all actual test vessels at test termination.

Guideline Criteria	Reported Information
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	3 uL were removed from each test vessel to confirm presence of methyl bromide. At 0 ant 48 hours, 2 mL were removed from each test chamber (gas-tight syringe) and transferred to autosampler.

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Static: ≤10% Flow-through: ≤5%	0 %
Recovery of Chemical	93-99%
Raw data included?	Yes

Mortality

Concentration (ppm)		Number of Organ-isms	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		2	24	48	96
Control	No Detect	20	0	0	0	
Solvent Control						
1.3	1.2	20	0	0	0	
2.2	2.2	20	0	0	1	
3.6	3.5	20	0	0	20	
6.0	5.8	20	0	13	20	
10	9.8	20	0	20	20	

Other Significant Results:

B. Statistical Results

Method: Binomial

48-hr LC_{50}: 2.6 ppm ai 95% C.I.: ____-____ ppm ai

Probit Slope: ____ NOEC: 1.2 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	2.6 (2.2 - 3.5) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	____ (____-____) ppm ai
Probit LC_{50} (95% C.I.)	____ (____-____) ppm ai
Probit Slope	
NOEC	1.2 ppm ai (observed)

15. REVIEWER'S COMMENTS:

This study is scientifically sound. The modified test design was acceptable and provided good results. The analytical procedure appeared to be adequate, however a qualified chemist should review the procedure to confirm its suitability. Methyl Bromide is moderately toxic to *Daphnia magna* with an LC50 of 2.6 mg a.i./L and an NOEC of 1.2 mg a.i./L.